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Contribution of dissolved free amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton

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ABSTRACT: We investigated the contribution of dissolved free amino acids (DFAA) and ammonium (NH_4^+) to the nitrogen demand (N-demand) of bacterioplankton in the subarctic Pacific and the Delaware Estuary. Bacteria were isolated from other microorganisms by gravity-filtration through 0.8 μm filters, and then bacterial abundance and nutrient concentrations were measured over time. During experiments lasting between 36 and 130 h, DFAA and NH_4^+ contributed 51 ± 45 % and 64 ± 54 % (n = 14) to the estimated N-demand, respectively. In 9 of the 14 experiments, DFAA and NH_4^+ contributed over 90 % of the estimated N-demand, implying that dissolved organic nitrogen aside from DFAA (e.g. dissolved combined amino acids) was not a significant source of nitrogen. Additions of glucose (0.1 to 1.0 μM) increased the contribution of NH_4^+ and DON other than DFAA to the bacterial N-demand. In most cases, measurements of amino acid and NH_4^+ uptake are sufficient for estimating bacterial nitrogen use.

INTRODUCTION

Heterotrophic bacteria can utilize a wide variety of organic and inorganic nitrogen sources for growth, including dissolved free amino acids (DFAA; Crawford et al. 1974), dissolved proteins and combined amino acids (Hollibaugh & Azam 1983, Coffin 1989), dissolved nucleic acids (Paul et al. 1989), and ammonium (Wheeler & Kirchman 1986). Little is known about the contribution of these nitrogenous compounds in supporting bacterial growth (N-demand) in marine systems. Previous studies have suggested that DFAA support a large fraction (20 to 100 %) of bacterial growth in sediments (Stanley et al. 1987), freshwaters (Jørgensen 1987), and marine waters (Billen & Fontigny 1987, Fuhrman 1990). Another potentially important nitrogen source for bacteria is ammonium. Bacteria can account for a substantial fraction (ca 50 %) of total NH_4^+ uptake in seawater (Wheeler & Kirchman 1986, Fuhrman et al. 1988, Kirchman et al. 1989). Tupas & Koike (1990) showed that NH_4^+ could support as much as 50 to 88 % of bacterial N-demand, even when large amounts of dissolved organic nitrogen (DON) were utilized.

Based on these previous studies, DFAA and NH_4^+ appear to account for most of the nitrogen needed for bacterial growth, although uptake rates of these 2 nitrogen pools have not been measured simultaneously. If DFAA and NH_4^+ do in fact support all of bacterial growth, then the role of other organic nitrogen becomes unclear. Aside from free and combined amino acids, there are no studies comparing uptake of organic nitrogen with bacterial growth.

Because NH_4^+ is the preferred nitrogen source for phytoplankton (e.g. Wheeler & Kokkinakis 1990), it is important to examine the control of NH_4^+ uptake by bacteria. Previous studies (Kirchman et al. 1989, 1990) have focused on the role of DFAA and dissolved organic carbon (DOC) in controlling NH_4^+ utilization. Kirchman et al. (1989) found that amino acids were preferred over NH_4^+ and that net NH_4^+ utilization only occurred when amino acid utilization was insufficient to meet N-demand. NH_4^+ utilization has also been found to be limited by the flux of DOC. Glucose additions to whole water stimulated NH_4^+ depletion in the subarctic Pacific, suggesting that NH_4^+ utilization by bacteria was limited by the supply rate of organic carbon (Kirchman et al. 1990).

This study examined the importance of DFAA and NH_4^+ for bacterial growth and the effect of glucose additions on DFAA and NH_4^+ uptake. At sites ranging from riverine to oceanic, DFAA and NH_4^+ supported >90 % of the bacterial growth in most experiments.

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DON other than DFAA (oDON, such as dissolved combined amino acids), calculated by difference, was not an important source of nitrogen in 9 of 14 experiments.

MATERIALS AND METHODS

Experiments were conducted in the subarctic North Pacific, the Delaware estuary, USA, and in Atlantic coastal waters near Delaware (Table 1). Surface or 10 m water was collected in Nisken bottles and the bacterial assemblage was isolated from grazers and autotrophs by gravity filtration through 0.8 μm × 142 mm Nuclepore filters (the bacterial size fraction). Size fractionation resulted in elevated dissolved primary amine (DPA) concentrations, ranging from 0 to 53 % above ambient (0 to 0.61 μM), probably the result of cell lysis during filtration. Because nearly all of this could be attributed to the 0.45 μm filtered water, 6 experiments were conducted using only 0.8 μm gravity-filtered water (Table 1). In the other 8 experiments, an equal portion of 0.45 μm Millipore filtered seawater was mixed with the bacterial size fraction. The water was placed in 4 l polypropylene bottles and incubated at surface seawater temperatures in the dark. Over time, samples were withdrawn and analyzed for inorganic nitrogen (NH_4^+, NO_3^- and NO_2^-), chlorophyll *a*, abundance of cyanobacteria, bacteria and bacterial grazers and dissolved primary amines (DPA). In 3 experiments (2 March 1988; 10 and 19 August 1988) dissolved free amino acids (DFAA) were measured along with DPA.

Inorganic nutrients were analyzed on a Spectraphysics autoanalyzer Chlorophyll *a* concentrations were determined after acetone extraction with 10 % DMSO (Stauffer et al. 1979). Cyanobacterial abundance was estimated from the number of autofluorescent cells using an epifluorescent microscope. Ammonium concentrations were determined by standard techniques (Grasshoff et al. 1983), with a detection limit of 0.05 μM and a coefficient of variation less than 10 %.

Dissolved primary amines were quantified by the ortho-phthaldialdehyde (OPA) method using a glycine standard as outlined in Parsons et al. (1984) and Kirchman et al. (1989). Fluorescence due to reaction of OPA with NH_4^+ was 3 % of glycine on a molar basis and was subtracted from total DPA fluorescence after NH_4^+ concentrations were independently measured (see above). Fluorescence from a variety of non-DFAA amine sources (peptides, proteins, alkyl amines and glucosamines) was less than 1 % of glycine on a molar basis and was small compared with DFAA fluorescence in our experiments. The coefficient of variation for triplicate samples was typically less than 8 %. Concentrations of DFAA were measured by reverse phase HPLC after derivatization with OPA (Lindroth & Mopper 1979, Kirchman et al. 1989). The gradient was essentially that used by Fuhrman & Bell (1985) with alpha-aminobutyric acid as the internal standard. DFAA samples were collected in muffled (500 °C for 2 h) 20 ml glass vials after filtration through 0.45 μm filters (Acrodiscs, Gelman Scientific), using clean techniques (Fuhrman & Bell 1985).

Net uptake rates of DPA and NH_4^+ were determined by linear regression analysis of the decrease in concentrations over a given sampling period. Coefficients of determination (r^2) varied from 0.847 to 0.996 ($n = 31$). Percentages of N-demand supported by DPA and NH_4^+ were determined by comparing individual uptake rates with N-demand (see below). Because N-demand was estimated independently of DPA or NH_4^+, it was possible that N-uptake exceeded N-demand (see 'Results'). N-demand supported by organic nitrogen

Table 1 Dates and locations of experiments (< 0.8 μm size fraction)

Date	Geographic location		Mixture[a]
3 Jun 1987	North Pacific	150° W, 50° N	+
10 Jun 1987	North Pacific	150° W, 51° N	+
21 Jun 1987	North Pacific	150° W, 51° N	+
29 Jul 1987	Coastal Atlantic, USA	74° W, 37° N	−
19 Sep 1987	North Pacific	150° W, 50° N	+
25 Sep 1987	North Pacific	150° W, 50° N	+
28 Sep 1987	North Pacific	150° W, 51° N	+
30 Sep 1987	North Pacific	150° W, 51° N	+
1 Oct 1987	North Pacific	150° W, 50° N	+
4 Nov 1987	Delaware Estuary, USA	Salinity 28 ‰	−
2 Mar 1988	Delaware River	Salinity 0 ‰	−
4 Aug 1988	North Pacific	150° W, 51° N	−
10 Aug 1988	North Pacific	150° W, 50° N	−
19 Aug 1988	North Pacific	150° W, 50° N	−

[a] Mixture: +, equal portions of 0.8 μm filtrate and 0.45 μm filtrate; −, 0.8 μm filtrate only

other than DPA (oDON) was estimated by subtracting DFAA and NH_4^+ uptake rates from N-demand rates and assuming the difference was oDON uptake.

Bacterial abundance was estimated using acridine orange epifluorescent microscopy (Hobbie et al. 1977). Growth rates were estimated from the slope of ln(bacterial abundance) vs time determined from a linear regression. Coefficients of determination (r^2) ranged from 0.932 to 0.991 (n = 33). Bacterial cell production (cell l^{-1} h^{-1}) was converted to N-demand (nM-N h^{-1}) using a conversion factor of 5.4 fg N $cell^{-1}$ (Lee & Fuhrman 1987). We estimated bacterial biovolume similarly to Lee & Fuhrman (1987). Briefly, we photographed acridine orange stained bacteria using Kodak T-max 100 black and white film. The photographic slides were projected and a minimum of 250 bacterial cells were measured. Biovolumes were calculated by assuming that cells were an average of true spheres and capped rods (spheroids; Lee & Fuhrman 1987). Measurements were calibrated using a photographed stage micrometer. Changes in biovolume were converted to N-demand using a conversion factor of 103 fgN μm^{-3} (Lee & Fuhrman 1987).

To test the effect of carbon supply on nitrogen utilization, 4 experiments had additional containers that received a single addition of glucose at the start of the experiment. Control bottles received no additions. Added concentrations varied from 0.1 to 1.0 µM and are reported with individual figures. To insure that low concentrations of NH_4^+ did not limit bacterial growth, NH_4^+ was added in 2 experiments along with the glucose addition. One experiment was conducted with only an NH_4^+ addition in order to test the effect of NH_4^+ additions on nitrogen utilization.

RESULTS

To determine whether DPA uptake was similar to DFAA uptake, DFAA and DPA concentrations were both measured in 3 experiments (2 March 1988 in the Delaware Estuary and 10 and 19 August 1988 in the subarctic Pacific). In the subarctic Pacific, DFAA and DPA concentrations were equal, within sampling error (Kirchman et al. 1989, Keil & Kirchman 1991). In the Delaware Bay, after accounting for the fluorescence of NH_4^+, DPA concentrations were generally 1.2 to 2.0-fold higher than DFAA estimates. A broad peak, which eluted immediately after the solvent front, was observed in HPLC analyses (Fig. 1). Amino acid standards did not co-elute with the unidentified compound and its peak area did not change during our experiments (Fig. 1). Despite differences in absolute fluorescent yields, DFAA and DPA concentrations decreased at a similar rate, indicating that changes in DPA were

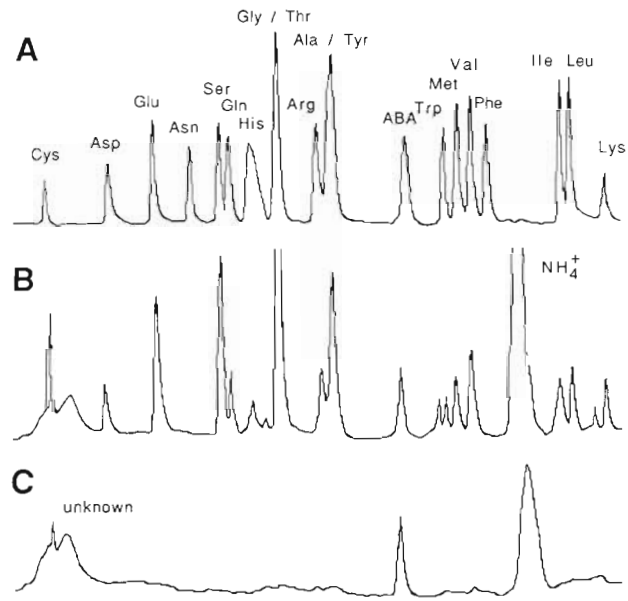

Fig. 1. (A) HPLC chromatograph of 5 pmol amino acid standards; ABA is α-aminobutyric acid, the internal standard. (B) Initial time point of 2 Mar 1988 experiment in the Delaware river; 100 pmol ABA. (C) Sample after 59 h incubation with <0.8 µm filtrate (bacterial size fraction); 100 pmol ABA. Analysis time was 25 min

equivalent to changes in DFAA in the Delaware estuary (Fig. 2).

Gravity-filtration was used to separate the bacterial assemblage from other microorganisms. In the subarctic Pacific, the 0.8 µm size fraction contained 97 % of the bacterial assemblage but only 0.5 % and 5.1 % of chlorophyll *a* and phaeopigments respectively (Kirchman et al. 1989). Similar results were obtained in the Delaware estuary (M. Hoch & R. Keil unpubl.). Abundance of intact autofluorescent cells was less than the detection limit of 10^4 cells ml^{-1}. In addition, bacterial grazers were not observed through the first 60 to 100 h of the experiments. As some evidence that bacterial

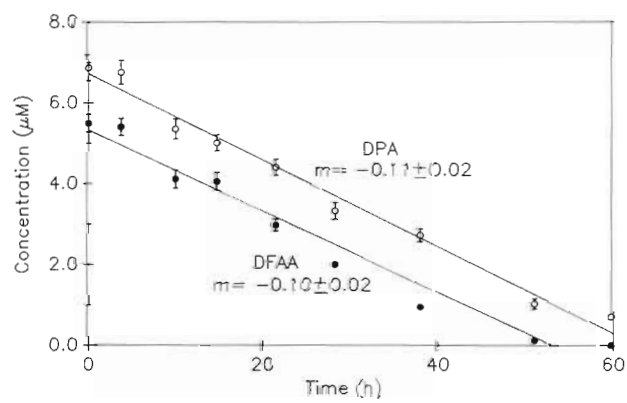

Fig. 2. Comparison of DPA and DFAA concentrations over time for the experiment of 2 Mar 1988. The slope (m ± SE) was calculated by linear regression

grazing was minimized, bacterial numbers increased through the first 60 h of all incubations (Fig. 3). All experiments were terminated when bacterial abundance stopped increasing (36 to 120 h).

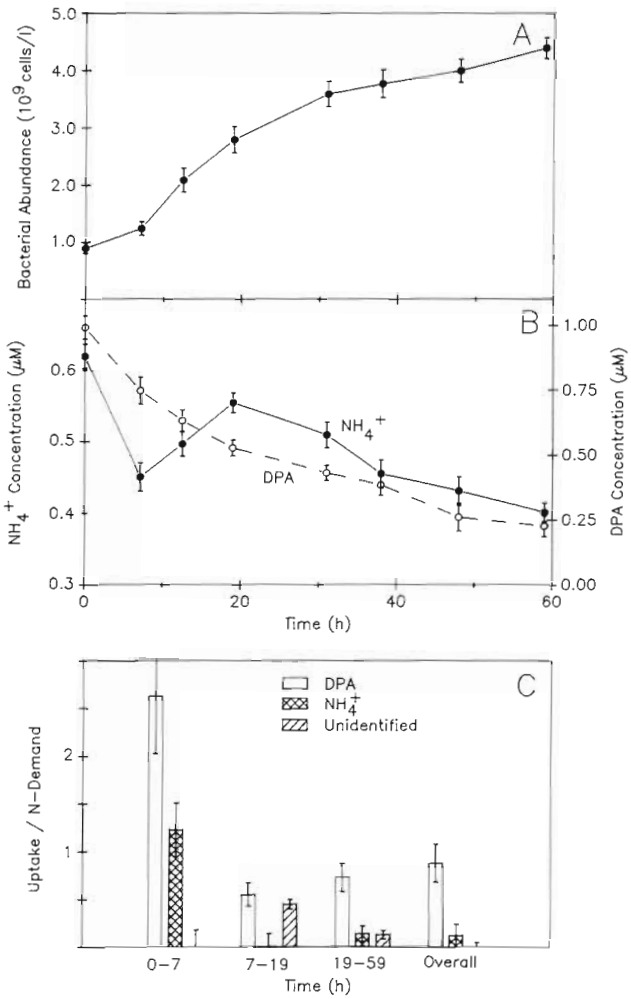

Fig. 3. Changes in (A) bacterial abundance, (B) NH_4^+ and DPA (= DFAA) concentrations over time for experiment of 21 June 1987. (C) N-demand supported by DPA (= DFAA), NH_4^+ and oDON (unidentified)

Amino acids and NH_4^+ as N-sources for bacterial growth

A total of 14 experiments were conducted in the subarctic Pacific and in the Delaware river, estuary and coastal waters (Table 1). Fig. 3 shows representative results observed in 9 of the 14 experiments. In these experiments NH_4^+ concentrations decreased for 6 to 12 h, before net increases were observed (Fig. 3). DPA concentrations decreased throughout all experiments (Fig. 3). In the remaining 5 experiments, NH_4^+ concentrations immediately increased at the start of experiments (Fig. 4). Aside from differences in bacterial growth rates and initial concentrations of nutrients, results from all sampling locations were similar. The percent of bacterial nitrogen demand supported by DPA, NH_4^+ and unidentified nitrogen for the experiments of 4 November 1987 and 21 June 1987 are shown in Figs. 3c & 4c, respectively.

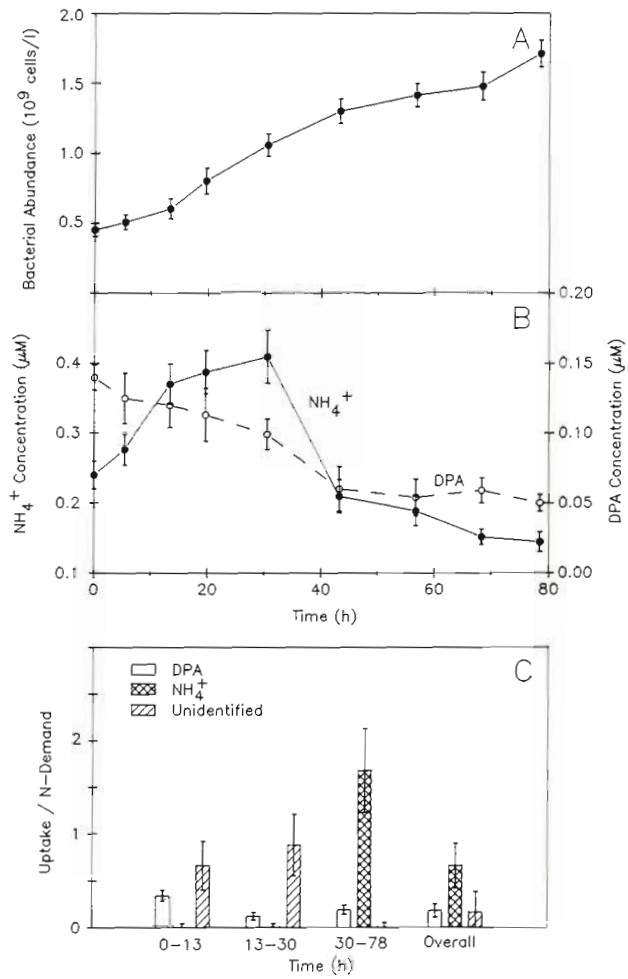

Fig. 4. Changes in (A) bacterial abundance, and (B) NH_4^+ and DPA concentrations over time for experiment of 4 Nov 1987. (C) N-demand supported by DPA, NH_4^+ and oDON

Over entire experiments, DPA and NH_4^+ supported 90 % or greater of the N-demand during 9 of 14 experiments (Table 2). The supply of DPA plus NH_4^+ did not meet N-demand in 5 experiments, and other nitrogen sources were apparently used (Table 2). Averaging the data from all 14 experiments, DPA and NH_4^+ uptake accounted for 96 % (± 62 standard deviation) and 72 % (± 69 %) respectively of the total nitrogen needed for bacterial growth during the initial hours (Table 2). Over entire experiments, DPA uptake

Table 2. Nitrogen budget during the initial hours of experiments (< 12 h) and for entire experiments (> 36 h)

Date	Time (h)	N-demand (nM h^{-1})	% N-demand supported by[a] DPA[b]	NH_4^+	oDON[c]
3 Jun 1987	11	7.6 ± 1.4	176 ± 43	98 ± 26	0
	96	7.4 ± 1.5	30 ± 7	65 ± 14	5
10 Jun 1987	12	3.9 ± 0.9	95 ± 25	0	5
	88	4.3 ± 1.0	14 ± 4	40 ± 11	46
21 Jun 1987	13	4.4 ± 1.0	34 ± 10	0	66
	78	5.0 ± 1.2	18 ± 8	66 ± 12	16
29 Jul 1987	12	9.3 ± 1.9	38 ± 10	0	62
	64	7.8 ± 1.6	123 ± 34	0	0
19 Sep 1987	14	11.2 ± 2.5	94 ± 27	0	6
	118	7.8 ± 1.7	33 ± 10	88 ± 23	0
25 Sep 1987	8	3.0 ± 0.5	80 ± 21	240 ± 52	0
	116	6.7 ± 1.3	27 ± 8	63 ± 16	10
28 Sep 1987	41	8.9 ± 1.9	83 ± 24	51 ± 12	0
	11	17.6 ± 4.0	35 ± 10	34 ± 9	31
30 Sep 1987	9	3.9 ± 0.9	100 ± 29	100 ± 25	0
	36	11.6 ± 2.5	21 ± 6	33 ± 9	46
1 Oct 1987	6	6.2 ± 1.2	27 ± 6	65 ± 15	8
	89	6.5 ± 1.4	14 ± 4	78 ± 21	8
4 Nov 1987	12	11.0 ± 2.6	246 ± 14	123 ± 37	0
	85	21.9 ± 4.7	89 ± 24	11 ± 3	0
2 Mar 1988	10	27.7 ± 5.9	55 ± 15	52 ± 15	0
	59	71.3 ± 17.7	146 ± 45	207 ± 65	0
4 Aug 1988	9	2.8 ± 0.6	71 ± 19	132 ± 34	0
	130	4.3 ± 0.9	60 ± 17	116 ± 31	0
10 Aug 1988	8	5.8 ± 1.4	172 ± 52	29 ± 8	0
	117	5.6 ± 1.4	20 ± 6	9 ± 3	71
19 Aug 1988	11	3.5 ± 1.0	66 ± 22	117 ± 40	0
	72	6.1 ± 0.8	95 ± 21	95 ± 19	0
Initial hrs (average ± SD):			96 ± 62	72 ± 69	11 ± 22
Overall:			51 ± 45	64 ± 54	18 ± 24

[a] % N-demand supported was calculated as (uptake/N-demand) × 100, where N-demand is estimated from the increase in bacterial abundance over time

[b] DPA = dissolved primary amines, equivalent to DFAA in these studies

[c] oDON = dissolved organic nitrogen other than DFAA. oDON % of N-demand was calculated as [N-demand – (DPA + NH_4^+ uptake)]/N-demand × 100

accounted for 51 % (± 45) and NH_4^+ uptake accounted for 64 % (± 54) of the N-demand. These results suggest that even after 100 h of incubation, DFAA and NH_4^+ still accounted for nearly all of the bacterial N-demand (Table 2).

Uptake of DPA and NH_4^+ did not satisfy N-demand during the inital 8 to 12 h in 2 of 14 experiments (21 June 1987 in the subarctic Pacific and 29 July 1987 in the Delaware estuary). In these 2 experiments, DPA and NH_4^+ accounted for only 36 % of the N-demand (Table 2). There were no measurable changes in the concentrations of NO_3^- and NO_2^- for any experiments (data not shown), suggesting that NO_3^- and NO_2^- did not contribute to bacterial N-demand. Thus, it appears that DON other than DFAA (oDON) was used to fulfill the remaining N-demand during the initial hours of these 2 experiments (Table 2). When considering entire experiments, oDON appears to have been used to support bacterial growth only 5 times (Table 2).

Our estimates of bacterial nitrogen demand were based solely on changes in bacterial abundance and assumed a constant biovolume. To determine the importance of changes in biovolume, we measured biovolumes during 2 experiments in the subarctic Pacific (21 June and 25 September 1987). Biovolumes were as low as 0.012 μm^3 at the beginning of experiments and increased to 0.096 μm^3 as bacterial abundance increased over time. Nitrogen demand was estimated using changes in both biovolume and abundance and compared to estimates assuming constant biovolume. In these experiments, N-demand estimates from biovolume and abundance did not differ signifi-

Table 3. Bacterial N-demand (± standard deviation) calculated from changes in bacterial abundance over time assuming constant biovolume or including measured biovolumes

Date	Experimental time (h)	N-demand (nM h^{-1}) Abundance	Biovolume
21 Jun 1987	0–6	1.99 ± 0.30	1.12 ± 0.67
	6–12	2.33 ± 0.35	1.58 ± 0.61
	12–20	6.05 ± 0.49	4.92 ± 1.21
	20–30	4.88 ± 0.80	6.39 ± 0.92
	30–44	3.68 ± 0.74	5.29 ± 1.34
	44–57	1.63 ± 0.16	1.65 ± 0.32
25 Sep 1987	0–8	2.99 ± 0.23	1.85 ± 1.09
	8–20	3.02 ± 0.13	2.48 ± 0.58
	20–31	3.02 ± 0.12	3.04 ± 0.55
	31–42	5.34 ± 0.23	4.88 ± 0.44
	42–54	4.83 ± 0.24	4.65 ± 0.52
	54–65	10.03 ± 0.42	11.09 ± 1.24
	65–79	11.71 ± 0.69	15.28 ± 2.72

cantly from N-demand estimated from changes in abundance alone (Table 3).

Effect of glucose and NH_4^+ additions

In 4 experiments, glucose was added to examine how an available carbon source affected nitrogen utilization. Uptake rates of DPA did not change when glucose was added (Fig. 5, see also Kirchman et al. 1990) but NH_4^+ was utilized immediately (no net regeneration was observed) and at a higher rate (10 to 500 % over controls) than in controls (Fig. 5). During one glucose experiment (25 September 1987), we observed that NH_4^+ uptake had stopped (NH_4^+ concentrations did not change) and we hypothesized that the bacterial assemblage had depleted its carbon supply. To test this, we added more glucose midway through the experiment (104 h) and observed that NH_4^+ uptake resumed and NH_4^+ concentrations began to decline again (Fig. 5).

The importance of DPA to the bacterial N-demand decreased during all phases of all 4 experiments in which glucose was added (Fig. 6). This decrease in DPA contribution resulted from the increased N-demand in the glucose treatment and the lack of a corresponding increase in amino acid uptake rates. This indicates that assimilation of DFAA nitrogen was maximal before the addition of the glucose (Figs. 5 & 6; Kirchman et al. 1990).

Over entire experiments when glucose was added, the NH_4^+ contribution to N-demand was not significantly different from controls because both NH_4^+ uptake rates and N-demand increased to the same degree when glucose was added. The overall contribution of oDON to N-demand increased in 3 of 4 experiments when glucose was added (Fig. 6). The effect was largest late in experiments (>60 h, 85 % increase) when both DPA and NH_4^+ uptake had decreased to low rates (Fig. 5).

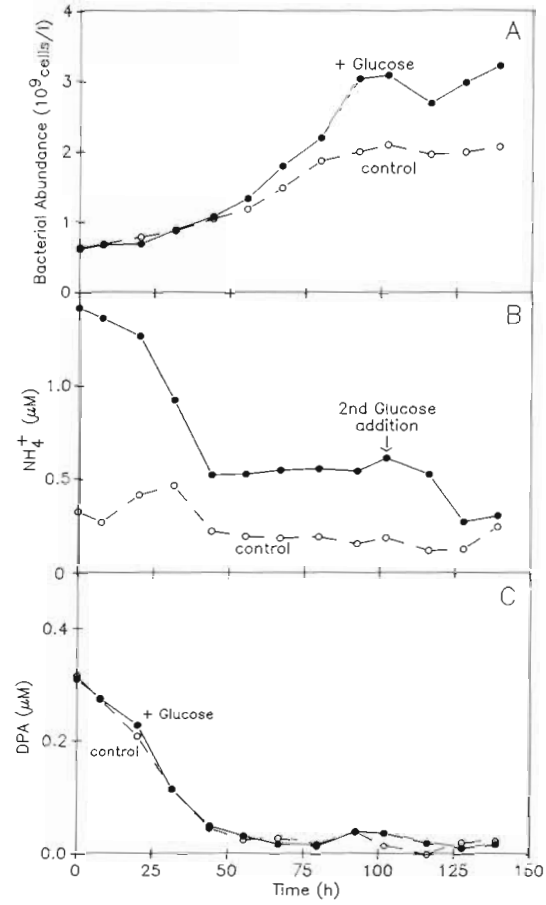

Fig. 5. Changes in (A) bacterial abundance, (B) NH_4^+ and (C) DPA concentrations over time after 1.0 μM glucose and NH_4^+ addition (25 Sep 1987)

We added NH_4^+ to 2 experiments along with glucose to insure that NH_4^+ concentrations were sufficient to observe changes in NH_4^+ uptake. A previous experiment (3 June 1987) showed that 1.0 μM NH_4^+ additions had no significant effect on the percent of nitrogen demand met by NH_4^+, nor on the uptake of DPA and NH_4^+ (data not shown).

DISCUSSION

During the initial hours of our experiments, when DPA and NH_4^+ concentrations were highest, uptake of DPA and NH_4^+ entirely supported the bacterial N-demand. As experiments progressed and concentrations of DPA and NH_4^+ decreased, DPA and NH_4^+

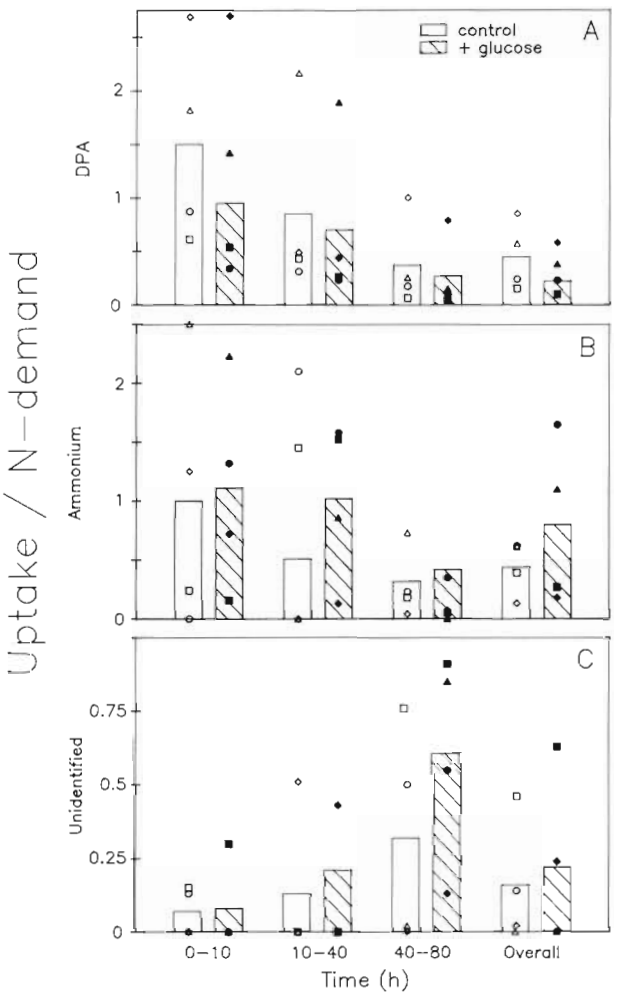

Fig. 6. Ratio of N-demand supported by DPA (= DFAA), NH_4^+ and unaccounted demand after glucose additions. (○, ●) 19 Sep 1987 (1.0 µM glucose and NH_4^+ addition); (□, ■) 25 Sep 1987 (1.0 µM glucose and ammonium addition); (△, ▲) 1 Oct 1987 (0.1 µM glucose addition); (◇, ◆) 4 Nov 1987 (0.25 µM glucose addition). Open symbols: control; closed symbols: glucose addition

still accounted for more than 90 % of the estimated bacterial N-demand in 9 of 14 experiments. Since DPA uptake was equal to DFAA uptake (discussed below), these results suggest that DFAA is the primary organic N-source supporting heterotrophic bacterial growth in the surface waters we sampled. This implies that DON other than DFAA (oDON) was not an important N-source in many of our experiments. We observed similar results in several diverse locations, including the subarctic Pacific, coastal Atlantic, Delaware estuary and Delaware river.

One important assumption is that changes in DPA were equivalent to changes in DFAA. In the subarctic Pacific, total DPA and DFAA concentrations were similar (within methodological error) during the experiments in which they were directly compared (this study, Kirchman et al. 1989, Keil & Kirchman 1991). After accounting for the low fluorescence of NH_4^+, fluorescence from other amines such as peptides and proteins apparently was minimal (Kirchman et al. 1989, Keil & Kirchman 1991). In a more comprehensive study, Delmas et al. (1990) observed similar results and concluded that DFAA and DPA measurements were equal when ammonium concentrations were no more than 10-fold greater than DFAA concentrations. In the Delaware estuary, after accounting for the fluorescence of NH_4^+, DPA concentrations were generally greater than DFAA estimates. This discrepancy appears to be partly the result of an unidentified non-amino acid peak which eluted early in our HPLC gradient. The compound(s) represented by this peak was not utilized during our experiments. When samples are hydrolyzed to measure dissolved combined amino acids (DCAA) this peak no longer appears, suggesting that the unidentified compound is heat-labile, unlike DFAA, and may represent some compound(s) in the DCAA pool. In spite of the unidentified compound, changes in DFAA nearly perfectly paralleled changes in DPA. We concluded that although concentrations were not equal in the Delaware estuary, DPA uptake rates were equal to DFAA uptake rates.

DFAA concentrations were unintentionally elevated during filtration prior to beginning experiments. Because 0.8 µm gravity-filtration did not artificially increase DFAA concentrations as much as 0.45 µm filtration did (average of 11 vs 38 % over in situ concentrations), experiments consisting of only 0.8 µm filtrate had DFAA concentrations close to or at ambient values. Results from these 2 experimental procedures indicate that DFAA were a significant source of bacterial nitrogen regardless of any unintentional release of DFAA during preparation of the experiments. In fact, DFAA actually supported a greater percentage of N-demand in experiments with less DFAA contamination (only 0.8 µm filtrate; Table 2). Furthermore, the contribution of DFAA to N-demand was not only high during the initial hours, but also late in experiments when DFAA concentrations decreased below that of the source water (Figs. 1 & 2). Overall, DFAA contributed half of the N-demand, despite low concentrations throughout most hours of the experiments.

The pulse of DFAA added during filtration may explain why uptake of DFAA and NH_4^+ often exceeded N-demand during the initial hours of experiments. The sudden elevation of DFAA concentrations may have caused DFAA uptake to increase without any corresponding increase in bacterial growth. Although subsequent regeneration of NH_4^+ later accounted for most of this 'excessive' uptake, at first it may seem hard to reconcile how bacteria could take up more nitrogen than they needed for biomass production. This suggests that

either our estimates of net uptake of DFAA and NH_4^+ were too high or N-demand estimates were too low.

There is no obvious reason to believe that net uptake of DFAA or NH_4^+ was overestimated. One possibility is that DON was lost to container walls, which would increase uptake estimates. However, concentrations of DFAA and NH_4^+ did not change in filter-sterilized controls (0.2 µm filtrate, data not shown). In addition, experiments have been conducted in containers ranging in size from 1 to 120 l (Kirchman et al. 1989, 1990, Hoch et al. 1989) and similar results have been observed regardless of the wide range of surface area to volume ratios of these containers.

Although it seems likely that N-demand was underestimated during the initial hours of experiments, our biovolume study indicates that we used a rather high conversion factor, at least in 2 experiments. The average initial biovolume in these experiments was 0.018 μm^3. If the Lee & Fuhrman (1987) estimate of 5.4 fgN $cell^{-1}$ is converted to a volumetric estimate using this initial biovolume, we calculate a conversion factor of 300 fgN μm^{-3}. This number is more than 3 times higher than any published value (Bratbak & Dundas 1984, Bjørnsen 1986, Lee & Fuhrman 1987, Nagata & Wanatabe 1990). Despite using a high conversion factor, our estimates of DFAA and NH_4^+ uptake often exceeded N-demand estimates. Apparently these compounds were not immediately used for cell production (increase in biovolume). This 'luxurious' uptake led to underestimates of total nitrogen uptake (N-demand). Eventually, in our experiments luxurious uptake was balanced by growth and mineralization to NH_4^+. Our experiments indicate that N-content $cell^{-1}$ may be quite variable, as suggested by Nagata (1986).

An indication that our estimates of the contribution of DFAA and NH_4^+ to bacterial growth are reasonable, even when these uptake rates exceed N-demand, is that similar results have been reported by other investigators. Studies in lakes have suggested that DFAA uptake can account from 28 to greater than 100 % of bacterial carbon requirements (Zehr et al. 1985, Jørgensen 1987). Billen & Fontigny (1987) consistently observed DFAA uptake rates that exceeded N-demand by as much as 600 %. Fuhrman et al. (1988) and Fuhrman (1990) also observed DFAA uptake that occasionally exceeded N-demand. Our approach was quite different from that of these previous investigators. We examined bacterial assemblages in <0.8 µm filtrates rather than unfractionated water for 3 reasons: (1) Bacterial production could be directly estimated from changes in cell abundance, avoiding methodological uncertainties with measuring production when grazers are present. (2) Without grazers and autotrophs there were no sources of DON. Therefore, we could directly compare changes in DFAA and NH_4^+ with increases in bacterial production without the problems associated with tracer techniques. (3) Finally, since grazers excrete NH_4^+, DFAA and probably other labile nitrogenous compounds (Nagata & Kirchman 1990), eliminating grazers enabled us to examine possible uptake of recalcitrant oDON as the concentrations of labile substrates decreased.

Our experiments suggest that in many instances oDON (including DCAA) is not an important N-source for bacterial growth. In contrast, other investigations have suggested that dissolved combined amino acids (DCAA) can be important in supporting bacterial growth (Hagström et al. 1984, Coffin 1989, Tupas & Koike 1990). Previous studies have focussed on protein turnover, one component of total DCAA utilization. Turnover rates of protein tracers appear to be similar to DFAA turnover rates, suggesting that protein is an important bacterial substrate (Hollibaugh & Azam 1983, Somville & Billen 1984, Coffin 1989). However, no study has directly compared protein utilization with N-demand (or bacterial production), although several studies have examined the turnover of radiolabelled proteins (Hollibaugh & Azam 1983, Coffin 1989) and fluorescent analogues of proteins (Somville & Billen 1984, Rosso & Azam 1987). Coffin (1989) calculated that DCAA uptake accounted for 51 % of total amino acid (DFAA + DCAA) uptake, but he did not compare DCAA uptake with N-demand. One shortcoming of experiments such as these is that the DCAA pool is probably not comprised solely of protein. Proteins comprise only 10 to 20 % of the total hydrolyzable amino acids in sediments (Mayer et al. 1986) and other studies suggest that the same is true in pelagic systems (Carlson et al. 1985, Keil & Kirchman 1991). Extrapolating from protein tracer experiments to the entire DCAA pool is likely to overestimate DCAA utilization in marine systems. Significant uptake of DCAA (and other oDON) may only occur when fluxes of labile DON are high, such as in the enriched cultures of Tupas & Koike (1990). While our data suggest that oDON is typically not important in supporting bacterial N-demand, our estimates of oDON uptake should be considered conservative. We assumed oDON was not utilized unless DFAA and NH_4^+ uptake was insufficient to support growth, an assumption which may not be true. We also measured net uptake, not absolute rates, which may be much higher.

Nevertheless, a rough calculation of oDON utilization based on other data also suggests that oDON is not an important N-source in the surface waters of marine systems. We can calculate a rate of oDON utilization from estimates of DON turnover and DON concentrations. Toggweiler (1989) recently estimated DON turnover time to be about 700 yr This turnover rate must be close to the turnover rate of oDON because

DFAA are a small fraction (< 5 %) of total DON (Sharp 1983). Based on new methodology Suzuki et al. (1985) suggested that DON concentrations are 30 to 40 μM-N in the North Pacific. These estimates of turnover time and DON concentration imply that the oDON utilization rate is about 0.16 nM d^{-1}. This rate is overestimated if DON concentrations are in fact lower Even this high oDON utilization rate is low compared with the average bacterial N-demand in oceanic waters such as the subarctic Pacific. In these waters, bacterial biomass is about 6 μgN l^{-1} and bacterial growth rate is roughly 0.05 d^{-1} (Kirchman & Keil unpubl.; Cho & Azam 1988, 1990 present similar data). These data imply a N-demand of 40 nM d^{-1}. In coastal waters where growth rates can be 1 d^{-1} (Ducklow & Kirchman 1983), the N-demand is 400 nM d^{-1}. Even with the lowest N-demand, oDON supplies only 0.4 % of N-demand and does not appear to be an important nitrogen source for bacterial growth.

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